

Lysozyme in water-acetone mixtures: Residual enzyme activity and preferential solvation

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Abstract

© 2017 by Nova Science Publishers, Inc. All rights reserved. Numerous benefits of employing enzymes in non-aqueous organic liquids exist, including catalysis of industrially-important synthetic reactions, suppression of undesirable side reactions caused by water, and high solubility of hydrophobic reagents. Residual enzyme activity was investigated to monitor stabilization/destabilization of hen egg-white lysozyme at low, intermediate, and high water content in acetone at 25°C. The advantages of our methods are as follows: 1. Residual enzyme activity curves can be determined in the entire range of water content in organic liquids. 2. Enzyme activity values can be measured at fixed reaction conditions. 3. Information about changes in the state of the catalytically-active sites can be obtained separately from other contributions (e.g., changes in the solvation of reagents and products in water - organic mixtures). Our results clearly demonstrate that the stabilization/destabilization of lysozyme depends significantly on water content in acetone. 1. At high water content, residual activity values are close to 100%. 2. A minimum on the residual activity curve was observed at intermediate water content. Acetone augments the irreversible inactivation of lysozyme in this region. 3. Lysozyme demonstrates significant residual activity in water-poor acetone.

Keywords

Acetone, Biocatalysis in water-organic mixtures, Enzyme activity, Lysozyme, Preferential solvation, Protein stability

References

- [1] Gregory, R. B. (1995). Protein hydration and glass transition behavior. In: Protein-Solvent Interactions (Gregory, R. B., ed.), Marcel Dekker, New York, 191-264.
- [2] Rupley, J. A., Careri, G. (1991). Protein hydration and function. *Adv. Protein Chem.* 41, 37-172.
- [3] Kuntz, I. D., Kauzmann, W. (1974). Hydration of proteins and polypeptides. *Adv. Protein Chem.* 28, 239-345.
- [4] Oleinikova, A., Smolin, N., Brovchenko, I., Geiger, A., Winter, R. (2005). Properties of spanning water networks at protein surfaces. *J. Phys. Chem. B.* 109, 1988-1998.
- [5] Durchschlag, H., Zipper, P. (2001). Comparative investigations of biopolymer hydration by physicochemical and modeling techniques. *Biophys. Chem.* 93, 141-157.
- [6] Sirotkin, V. A., Khadiullina, A. V. (2013). Gibbs energies, enthalpies, and entropies of water and lysozyme at the inner edge of excess hydration. *J. Chem. Phys.* 139, 075102/1-075102/9.
- [7] Sirotkin, V. A., Khadiullina, A. V. (2014). A study of the hydration of ribonuclease A using densitometry: Effect of the protein hydrophobicity and polarity. *Chem. Phys. Lett.* 603, 13-17.
- [8] Privalov, P. L., Crane- Robinson, C. (2017). Role of water in the formation of macromolecular structures. *Eur. Biophys. J.* 46, 203-224.

- [9] Xu, M., Ermolenkov, V. V., Uversky, V. N., Lednev, I. K. (2008). Hen egg white lysozyme fibrillation: A deep-UV resonance Raman spectroscopic study. *J. Biophoton.* 1, 215-229.
- [10] Rezaei-Ghaleh, N., Zwecktetter, M., Morshedi, D., Ebrahym-Habibi, A., Nemat-Gorgani, M. (2008). Amyloidogenic potential of α -chymotrypsin in different conformational states. *Biopolymers* 91, 28-36.
- [11] Kijima, T., Yamamoto, S., Kise, H. (1996). Study of tryptophan fluorescence and catalytic activity of α -chymotrypsin in aqueous organic media. *Enz. Microb. Technol.* 18, 2-6.
- [12] Sirotkin, V. A., Hüttl R., Wolf, G. (2005). Thermochemical investigations of hydrolysis of p-nitrophenyl acetate in wateracetonitrile mixtures, *Thermochim. Acta* 426, 1-6.
- [13] Khmel'nitsky, Yu., Mozhaev, V. V., Belova, A. B., Sergeeva, M. V., Martinek, K. (1991). Denaturation capacity: A new quantitative criterion for selection of organic solvents as reaction media in biocatalysis. *Eur. J. Biochem.* 198, 31-41.
- [14] Partridge, J., Moore, B. D., Halling, P. J., (1999) α -Chymotrypsin stability in aqueous-acetonitrile mixtures: Is the native enzyme thermodynamically or kinetically stable under low water conditions? *J. Mol. Cat. B: Enzym.* 6, 11-20.
- [15] Griebenow, K.; Klivanov, A. M. (1996). On protein denaturation in aqueous-organic mixtures but in pure organic solvents. *J. Amer. Chem. Soc.* 118, 11695-11700.
- [16] Klivanov, A. M. (2001). Improving enzymes by using them in organic solvents, *Nature* 409, 241-246.
- [17] Halling, P. J. (2004). What can we learn by studying enzymes in nonaqueous media? *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 359, 1287-1297.
- [18] Lousa, D., Baptista, A. M., Soares, C. M. (2013). A molecular perspective on nonaqueous biocatalysis: Contributions from simulation studies. *Phys. Chem. Chem. Phys.* 15, 13723-13736.
- [19] Carrea, G., Riva, S. (2000). Properties and synthetic applications of enzymes in organic solvents, *Angew. Chem. Int. Ed.* 39, 2226-2254.
- [20] Micaelo, N. M., Soares, C. M. (2007). Modeling hydration mechanisms of enzymes in nonpolar and polar organic solvents, *FEBS J.* 274, 2424-2436.
- [21] Clark, D. S. (2004). Characteristics of nearly dry enzymes in organic solvents: implications for biocatalysis in the absence of water, *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 359, 1299-1307.
- [22] Serdakowski, A. L., Dordick, J. S. (2007). Enzyme activation for organic solvents made easy, *Trends Biotechnol.* 26, 54-48.
- [23] Rariy, R., Klivanov, A. M. (1997). Correct protein folding in glycerol, *Proc. Natl. Acad. Sci. U.S.A.* 94, 13520-13523.
- [24] Timasheff, S. N. (2002). Protein-solvent preferential interactions, protein hydration, and the modulation of biochemical reactions by solvent components. *Proc. Natl. Acad. Sci. USA.* 99, 9721-9726.
- [25] Arakawa, T., Kita, Y., Timasheff, S. N. (2007). Protein precipitation and denaturation by dimethyl sulfoxide. *Biophys. Chem.* 131, 62-70.
- [26] Gekko, K., Ohmae, E., Kameyama, K., Takagi, T. (1998). Acetonitrile-protein interactions: Amino acid solubility and preferential solvation. *Biochim. Biophys. Acta* 1387, 195-205.
- [27] Kovrigin, E. L., Potekhin, S. A. (2000). On stabilizing action of protein denaturants: Acetonitrile effect on stability of lysozyme in aqueous solutions. *Biophys. Chem.* 83, 45-59.
- [28] Shimizu, S., Matubayasi, N. (2014). Preferential solvation: Dividing surface vs excess numbers. *J. Phys. Chem. B.* 118, 3922-3930.
- [29] Auton, M., Bolen, D. W., Rosgen, J. (2008). Structural thermodynamics of protein preferential solvation: Osmolyte solvation of proteins, amino acids, and peptides. *Proteins* 73, 802-813.
- [30] Casassa, E. F., Eisenberg, H. (1964). Thermodynamic analysis of multicomponent solutions. *Adv. Protein Chem.* 19, 287-395.
- [31] Tanford, C. (1969.) Extension of theory of linked functions to incorporate effects of protein hydration. *J. Mol. Biol.* 39, 539-544.
- [32] Schellman, J. A. (1987). Selective binding and solvent denaturation. *Biopolymers* 26, 549-559.
- [33] Arakawa, T., Bhat, R., Timasheff, S. N. (1990). Why preferential hydration does not always stabilize the native structure of globular proteins. *Biochem.* 29, 1924-1931.
- [34] Smith, P. E. (2006). Equilibrium dialysis data and the relationships between preferential interaction parameters in biological systems in terms of Kirkwood-Buff integrals. *J. Phys. Chem. B* 110, 2862-2868.
- [35] Parsegian, V. A., Rand, R. P., Rau, D. C. (2000). Osmotic stress, crowding, preferential hydration, and binding: A comparison of perspectives. *Proc. Natl. Acad. Sci. U.S.A.* 97, 3987-3992.
- [36] Kirby Hade, E. P., Tanford, C. (1967). Isopiestic compositions as a measure of preferential interactions of macromolecules in twocomponent solvents. Application to proteins in concentrated aqueous cesium chloride and guanidine hydrochloride. *J. Amer. Chem. Soc.* 89, 5034-5040.

- [37] Kamiyama, T., Liu, H. L., Kimura, T. (2009). Preferential solvation of lysozyme by dimethyl sulfoxide in binary solutions of water and dimethyl sulfoxide. *J. Therm. Anal. Cal.* 95, 353-359.
- [38] Reisler, E., Haik, Y., Eisenberg, H. (1977). Bovine serum albumin in aqueous guanidine hydrochloride solutions. Preferential and absolute interactions and comparison with other systems. *Biochem.* 16, 197-203.
- [39] Izumi, T., Yoshimura, Y., Inoue, H. (1980). Solvation of lysozyme in water/dioxane mixtures studied in the frozen state by NMR spectroscopy. *Arch. Biophys. Biochem.* 200, 444-451.
- [40] Fersht, A. (1999). *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding*; Freeman & Co: New York.
- [41] Lehninger, A. L.; Nelson, D. L.; Cox, M. M. (1993). *Principles of Biochemistry*; Worth: New York.
- [42] Perrin, D. D., Armarego, W. L. F., Perrin, D. R. (1980). *Purification of Laboratory Chemicals*, Oxford: Pergamon Press.
- [43] Borisover, M. D., Sirotkin, V. A., Solomonov, B. N. (1995). Isotherm of water sorption by human serum albumin in dioxane: Comparison with calorimetric data. *J. Phys. Org. Chem.* 8, 84-88.
- [44] Sirotkin, V. A., Borisover, M. D., Solomonov, B. N. (1995). Heat effects and water sorption by human serum albumin on its suspension in water-dimethyl sulfoxide mixtures. *Thermochim. Acta*, 256, 175-183.
- [45] Sirotkin, V. A., Kuchierskaya, A. A. (2017). Preferential solvation/hydration of α -chymotrypsin in water-acetonitrile mixtures. *J. Phys. Chem. B.* 121, 4422-4430.
- [46] Sirotkin, V. A., Kuchierskaya, A. A. (2017). Lysozyme in water-acetonitrile mixtures: Preferential solvation at the inner edge of excess hydration. *J. Chem. Phys.* 146, 215101-8.
- [47] Sirotkin, V. A., Kuchierskaya, A. A. (2017). α -Chymotrypsin in water-ethanol mixtures: Effect of preferential interactions. *Chem. Phys. Lett.* 689, 156-161.
- [48] Sirotkin, V. A., Kuchierskaya, A. A. (2017). α -Chymotrypsin in water-acetone and water-dimethyl sulfoxide mixtures: Effect of preferential solvation and hydration. *Proteins: Functions, Structure and Bioinformatics*, 85, 1808-1819.
- [49] Sirotkin V. A., Korolev D. V. (2005). Effect of acetonitrile on the hydration of human serum albumin films: a calorimetric and spectroscopic study. *Thermochim. Acta*, 432, 246-253.
- [50] Atkins, P. W. (2006). *Physical Chemistry*. 8 ed. Oxford: Oxford University Press.
- [51] Prausnitz, J. M. (1969). *Molecular Thermodynamics of Fluid-Phase Equilibria*. N. J.: Prentice-Hall, Inc., Engelwood Cliffs.
- [52] Belousov, V. P., Panov, M. Y. (1994). *Thermodynamic properties of aqueous solutions of organic substances*. Boca Raton, Fla.: CRC Press.
- [53] Kogan, V. B., Fridman, B. N., Kafarov, V. V. (1966). *Liquid-Vapor Equilibrium*. Moscow: Nauka.
- [54] Bell, G., Janssen, A. E. M., Halling, P. (1996). Water activity fails to predict critical hydration level for enzyme activity in polar organic solvents: Interconversion of water concentrations and activities. *Enzym. Microb. Technol.* 20, 471-476.
- [55] Bull, H. B. (1944). Adsorption of water vapor by proteins. *J. Amer. Chem. Soc.* 66, 1499-1507.
- [56] Luscher-Mattli, M., Ruegg, M. (1982). Thermodynamic functions of biopolymer hydration. I. Their determination by vapor pressure studies, discussed in an analysis of the primary hydration process. *Biopolymers* 21, 403-418.
- [57] Luscher-Mattli, M., Ruegg, M. (1982). Thermodynamic functions of biopolymer hydration. II. Enthalpy-entropy compensation in hydrophilic hydration process. *Biopolymers* 21, 419-429.
- [58] Bone, S. (1987). Time-domain reflectometry studies of water binding and structural flexibility in chymotrypsin. *Biochem. Biophys. Acta.* 916, 128-134.
- [59] Hnojewy, W. S., Reyerson, L. H. (1961). Further studies on the sorption of H₂O and D₂O vapors by lysozyme and the deuterium-hydrogen exchange effect. *J. Phys. Chem.* 65, 1694-1698.
- [60] Sirotkin, V. A., Khadiullina, A. V. (2011). Hydration of proteins: excess partial enthalpies of water and proteins. *J. Phys. Chem. B.* 115, 15110-15118.
- [61] Sirotkin, V. A., Komissarov, I. A., Khadiullina, A. V., (2012). Hydration of proteins: excess partial volumes of water and proteins, *J. Phys. Chem. B.* 116, 4098-4105.
- [62] Sirotkin, V. A. (2005). Effect of dioxane on the structure and hydration-dehydration of α -chymotrypsin as measured by FTIR spectroscopy. *Biochim. Biophys. Acta.*, 1750, 17-29.
- [63] Kocherbitov, V., Arnebrant, T. (2010). Hydration of Lysozyme: The Protein-Protein Interface and the Enthalpy-Entropy Compensation. *Langmuir*, 26, 3918-3922.
- [64] Foss, J. G., Reyerson, L. H. (1958). The sorption of water vapor by lyophilized ribonuclease. *J. Phys. Chem.* 62, 1214-1216.
- [65] Amberg, C. H. (1957) Heats of adsorption of water vapor on bovine serum albumin. *J. Amer. Chem. Soc.* 79, 3980-3984.
- [66] Almog, R., Schrier, E. E. (1978). The dependence of the heat of solution of lyophilized solid ribonuclease A on water content. *J. Phys. Chem.* 82, 1701-1702.

- [67] Smith, A. L., Shirazi, H. M., Mulligan, S. R. (2002). Water sorption isotherms and enthalpies by lysozyme using the quartz crystal microbalance/heat conduction calorimeter. *Biochim. Biophys. Acta*, 1594, 150-159.
- [68] Kocherbitov, V., Arnebrant, T., Söderman, O. (2004). Lysozymewater interactions studied by sorption calorimetry. *J. Phys. Chem. B*, 108, 19036-19042.